

THE NUTRITIONAL RESPONSE OF TWO PENAEID SPECIES  
TO VARIOUS LEVELS OF SQUID MEAL  
IN A PREPARED FEED<sup>1</sup>

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ABSTRACT

Several pelletized feeds containing 30-35% total protein were tested on two penaeid species, *Penaeus stylirostris* and *P. setiferus*. Alginate bound test diets varied in protein composition but all diets contained about 30% sun-dried shrimp meal. Following initial testing with diets containing up to 13% squid meal, further test diets varied in percentage of squid meal between 0 and 12.7, and in  $\alpha$ -soy flour between 0 and 12.4. One test diet replaced these components with 12.5% brewer's yeast.

Conversion rates, survival, and growth were determined during a 3-week period, both in the two species and in two sizes of *P. stylirostris*.

Comparisons of the various diets between the two species suggest that the presence of 5-6% squid meal is advantageous in feeds of about 30-35% total protein.

INTRODUCTION

Although squid meal has been used as a protein source in feeds prepared for *Penaeus japonicus* (Kitabayashi 1971a,b; Deshimaru and Shigeno 1972), few attempts have been made to define an optimal level of this component for species less dependent on animal protein. Earlier studies (Fenucci and Zein-Eldin 1976; Fenucci 1977) suggested an optimal level of squid meal between 5 and 15% for juvenile *P. aztecus* and probably 5% for *P. setiferus*.

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The present experiments evaluate feeds containing percentages of squid meal less than 13% in rations for the two species, *P. setiferus* and *P. stylirostris*. Both the percentage of squid meal and the ratio of this ingredient to the vegetal protein source,  $\alpha$ -soy, were varied. In a further attempt to produce an economical feed, yeast was substituted for a portion of the vegetal protein.

#### MATERIALS AND METHODS

Glass aquaria of 60-liter capacity were prepared with undergravel filter beds consisting of oyster shell and sand (Zein-Eldin and Meyers 1973). Natural sea water used in experimentation was passed through a 5- $\mu$  filter and then through a quartz ultraviolet sterilizer. Aquaria were placed in controlled temperature rooms maintained at  $28 \pm 1^\circ\text{C}$ , with a light cycle of 12 hours on and 12 hours off.

The shrimp were fed one of the several pelletized feeds given in Table 1. Feeds were prepared using the ingredients and techniques as described by Fenucci and Zein-Eldin (1979). Initial feeding was approximately 5% of the biomass per day in two feedings. Uneaten feed was removed daily, dried, and weighed to determine the exact weight of the food eaten. The amount was adjusted daily so the animals were provided as much food as they could eat. Exuviae and dead shrimp were recorded and removed daily.

Three experiments were performed, one with *P. setiferus* (1.5 g) and two with *P. stylirostris* (0.7 and 4.1 g). All animals were laboratory hatched and reared penaeids. One group of *P. stylirostris* (0.7 g) was the product of a single female, mated in captivity, reared to postlarvae at the Galveston Laboratory, and transferred to ponds in Corpus Christi before return for laboratory experiments.

Experimental animals were weighed individually to the nearest 0.01 g, both initially and at the end of the experiment. Mean weights and SEM were calculated using all the individual weights of animals fed a diet rather than from group means.

Water temperature ranged from 27 to  $30.5^\circ\text{C}$  and the salinity between 20 and 22 ppt; in the second experiment (0.7 g animals), temperature varied from 27 to  $31^\circ\text{C}$  and the salinity from 27 to 30 ppt.

The feed conversion ratios (FCR) were calculated as amount of feed eaten divided by the increase in biomass. Rates calculated using corrected biomass values would be smaller (more efficient) than those reported here.

#### DESIGN OF FEEDS

Diet K, containing 31.5% shrimp meal and described in Fenucci and Zein-Eldin (1976), was used as a standard in all the experiments. In two experiments, a commercially available marine ration, ground and re-bound with 1% alginate and 1% sodium hexametaphosphate, served as an additional control as diet 11-B (Table 1). Other feeds to be tested were divided in two groups. The first grouping, 1 to 4 (Table 1) varied the proportions of squid meal and  $\alpha$ -soy, maintaining total protein, shrimp meal, fish meal, and fish solubles constant. Diet 1 contains only squid meal and Diet 3 only  $\alpha$ -soy. Diet 2 contains equal ratios of

the two components while diet 4 has one part of squid to three parts  $\alpha$ -soy. The standard K, however, has 5 parts squid to 3 of  $\alpha$ -soy.

Table 1. Percentage Composition of Formulated Feeds

Component	Diet number									
	1	2	3	4	K	18	22	23	24	29
Shrimp meal	28.4	28.4	28.4	28.4	31.5	28.4	31.5	31.5	31.5	28.4
Squid meal	12.7	6.4	--	3.2	5.0	6.4	5.0	5.0	5.0	5.0
$\alpha$ -soy	--	6.2	12.4	9.3	3.0	--	3.0	3.0	3.0	3.0
Fish meal	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Fish solubles	2.0	2.0	2.0	2.0	2.0	2.0	--	2.0	2.0	2.0
Rice bran	42.2	42.5	42.7	42.6	44.0	36.2	46.0	45.5	45.5	47.1
Vitamin mix <sup>a</sup>	2.0	2.0	2.0	2.0	2.0	2.0	2.0	0.5	0.5	2.0
Lecithin	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Alginate	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Na hexameta-phosphate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Yeast	--	--	--	--	--	12.5	--	--	--	--
Protein <sup>b</sup>	34.7	33.8	34.9	36.5	31.2	35.0	29.6	32.4	35.8	32.2

Note: Diet 11-B consists of a commercially available ration reground and bound using 1% alginate and 1% Na hexametaphosphate, and contains 19.4% protein.

<sup>a</sup>Source of components as in Zein-Eldin and Meyers (1973) except for Vitamin Mix--AIN Vitamin Mixture 76<sup>a</sup> of ICN Nutritional Biochemicals containing no ascorbic acid, choline or inositol. Diet 24 replaced AIN vitamin mix with mixture containing these three components as supplements.

<sup>b</sup>Determined by Kjeldahl.

In the second feed sequence, Diet 18 replaced both the  $\alpha$ -soy in Diet 2 and 6% of the rice bran with yeast protein (12.5%), while holding shrimp meal at 28.4%. Diet 29 also contains 28.4% shrimp meal, but has the squid:soy ratio of the standard, K. Diets 22-24 evaluated the effect of fish solubles (Diet 22) and a lower level of vitamins (0.5%) (Diets 23 and 24), as compared to the standard diet, K.

#### RESULTS

##### *Penaeus setiferus*

Diets 1, 2, 3, 4, K, 18, and 11-B were tested in four replicate tanks of 10 animals. The initial mean weight ranged from 1.50 to 1.53 g (Table 2). Diets 18 and K produced the greatest increase in mean weight, 1.58 and 1.44 g, respectively. The smallest increases after 28 days of feeding occurred among animals fed diet 11-B (0.51 g). Diet K also provided the best percentage increase of biomass, 97.2%, followed by Diets 2, 1, and 18, all of which contained squid meal. The smallest increase in percentage of biomass, only 29.9%, was observed among the shrimp given the prepared commercial ration. These animals also ingested only 91 g of feed compared to 175 g for shrimp fed K.



Survival was generally excellent in all groups, ranging from 87.5% to 100%, while the conversion ratios ranged from 3.0 in groups fed Diet K to 5.0 for ration 11-B.

Table 2. Growth and Survival of *P. setiferus* Fed Various Rations for 28 Days. Combined results of 4 replicate tanks, each containing 10 animals. Mean weight  $\pm$  SEM, calculated from individual measurements.

Diet	Mean wt (g)		Biomass (g) Final	Survival (%)	Amount fed (g)	Conversion rate	Change in biomass (%)
	Initial	Final					
1	1.51 $\pm$ 0.060	2.87 $\pm$ 0.077	108.97	95.0	162.6	3.3	80.7
2	1.51 $\pm$ 0.089	2.84 $\pm$ 0.083	113.44	100.0	164.6	3.1	87.5
3	1.50 $\pm$ 0.069	2.71 $\pm$ 0.132	95.00	87.5	139.9	4.0	58.7
4	1.53 $\pm$ 0.055	2.77 $\pm$ 0.104	102.31	92.5	152.4	3.7	67.6
K	1.49 $\pm$ 0.057	2.93 $\pm$ 0.112	117.25	100.0	174.9	3.0	97.2
18	1.53 $\pm$ 0.070	3.03 $\pm$ 0.091	109.01	90.0	164.5	3.4	79.3
11-B	1.52 $\pm$ 0.077	2.02 $\pm$ 0.073	78.92	97.5	90.6	5.0	29.9

#### *Penaeus stylirostris*

Two experiments were performed. In the first one, Diets 1, 2, 3, 4, and K were tested in duplicate tanks of 10 animals. The initial mean weight ranged from 4.11 to 4.27 g (Table 3). After 14 days, shrimp fed Diet 2 showed both the best increase in mean weight and percentage of biomass (1.77 g and 34.7%), followed by Diet 4 (1.45 g increase in mean weight and 28.4% increase in biomass). Survival was generally excellent. Feed consumed was again greatest for the group fed Diet K. The conversion ratios ranged from 2.59 for Diet 2 to 5.6 with Diet 1.

Table 3. Growth and Survival of Juvenile *P. stylirostris* Given Various Rations for 14 Days. Combined results for 2 replicate tanks of 10 animals. Mean weight  $\pm$  SEM calculated using individual measurements.

Diet	Mean wt (g)		Biomass (g) Final	Survival (%)	Amount fed (g)	Conversion rate	Change in biomass (%)
	Initial	Final					
1	4.11 $\pm$ 0.278	5.31 $\pm$ 0.350	95.5	90	75.2	5.6	16.3
2	4.24 $\pm$ 0.324	6.01 $\pm$ 0.395	111.2	95	76.2	2.6	34.7
3	4.16 $\pm$ 0.327	5.08 $\pm$ 0.341	96.6	95	68.1	5.1	16.1
4	4.18 $\pm$ 0.288	5.69 $\pm$ 0.292	108.2	95	69.8	2.9	29.6
K	4.27 $\pm$ 0.326	5.26 $\pm$ 0.342	99.9	95	83.1	4.7	21.3

In the second experiment with small juveniles, Diets 1, 2, 3, 4, K, 18, 22, 23, 24, 29, and 11-B were tested in triplicate tanks. Initial mean weight varied between 0.70 and 0.72 g with 15 animals in each tank. Final measurements were made after 18 days (Table 4). Best increase in mean weight was recorded among shrimp given Diets 1 and 29, with mean increases of 0.94 and 0.98 g after 18 days. This was followed by Diets K, 23, 24, and 18, all of which recorded an approximate increase of 0.7 g. The poorest recorded mean weight increase occurred in animals fed the commercial ration 11-B, only 0.51 g. The greatest increase in biomass was observed in animals fed Diet 29, 120.8%, followed by Diet K with 96.9%. All other feeds produced less than 75% increase in biomass largely because of poor survival. The exception to this was Diet 11-B, in which 91% of the shrimp survived, but there was only a 57% increase in biomass. Ingestion rates varied, with most food eaten by animals fed Diets 29, 1, K, and 24. The best conversion ratios were recorded for Diets 29 (2.8), K (3.3), and the commercial ration 11-B (3.9).

Both reduction in vitamin level (23, 24) and removal of fish solubles (22) from the formulation resulted in decreased survival as compared to the standard, K.

Table 4. Growth and Survival of *P. stylirostris* (0.7 g initial weight) Fed Various Rations for 18 Days. Combined results from 3 replicate tanks, each containing 15 animals. Mean weight  $\pm$  SEM calculated from individual measurements.

Diet	Mean wt (g)		Biomass (g) Final	Survival (%)	Amount fed (g)	Conversion rate	Change in biomass (%)
	Initial	Final					
1	0.72 $\pm$ 0.019	1.66 $\pm$ 0.052	56.42	76	106.3	4.4	74.6
2	0.72 $\pm$ 0.018	1.40 $\pm$ 0.049	36.42	58	75.1	18.5	12.5
3	0.71 $\pm$ 0.017	1.38 $\pm$ 0.050	42.79	69	71.1	6.6	33.7
4	0.77 $\pm$ 0.019	1.43 $\pm$ 0.042	35.86	56	71.7	26.8	8.0
18	0.72 $\pm$ 0.019	1.49 $\pm$ 0.041	49.35	73	98.1	5.8	52.0
K	0.71 $\pm$ 0.016	1.57 $\pm$ 0.042	62.93	89	103.8	3.3	96.9
11-B	0.70 $\pm$ 0.020	1.21 $\pm$ 0.040	49.64	91	70.0	3.9	56.9
22	0.71 $\pm$ 0.016	1.48 $\pm$ 0.055	44.37	67	93.5	7.5	38.8
23	0.71 $\pm$ 0.019	1.52 $\pm$ 0.044	53.20	78	97.2	4.6	67.1
24	0.72 $\pm$ 0.019	1.61 $\pm$ 0.043	51.60	71	100.4	5.2	60.2
29	0.71 $\pm$ 0.019	1.69 $\pm$ 0.040	70.83	93	106.9	2.8	120.8

#### DISCUSSION

*P. setiferus* diets containing squid meal (1, 2, 4, K, and 18) produced better growth as a group than those without this component (3, 17, and 11-B). Among the squid-containing feeds, there was a further slight separation, Diets K, 2, 1, and 18 being somewhat more efficient than Diet 4. The latter feed contains a higher percentage of soy flour (9.4%) than any of the others, and this diet, together with 3 (containing 12.4% soy, Table 1), yielded less biomass over the term of the experiment.

There are two possible inferences, either that 5-6% squid meal is optimal for *P. setiferus*, or that the ratio of 1.7 parts squid to 1 part soy is optimal for this species. These results are consistent with those reported by Fenucci (unpublished FAO report, 1977) in which Diet K with 5% squid meal produced the best growth for *P. setiferus*. A larger percentage of squid meal, 15%, appeared to be more satisfactory for *P. aztecus* (Fenucci and Zein-Eldin 1976). Kittaka (1976) also reported that a diet with squid and *Euphasia* supplemented with trash fish showed good results when used with *P. setiferus*. Much higher quantities of squid meal are used in diets reported as successful in the Japanese species, *P. japonicus*, with feeds containing more than 45% of this component (Kitabayashi et al. 1971a,b; Deshimaru and Shigeno 1972). Growth with feed 18, in which the expensive soy flour has been replaced by dried brewer's yeast, suggests that mean weight was not significantly reduced, while feed cost has been cut--an important economic advantage. Nevertheless, in the series of experiments with *P. setiferus*, Diet K remained the feed of choice (Fenucci, unpublished FAO report, 1977).

Results with *P. stylirostris* appear to depend somewhat upon the size of animals tested. Among the larger animals (4 g initial weight), Diets 2 and 4 were superior to the others tested, with Diets K and 3 falling below. In this group, it appeared that best growth was obtained when ratios of squid and soy were equal, or soy was in greater quantity. Because of the short term of the experiment and the relatively large initial size of the shrimp, speculation concerning these points should not be too great.

*P. stylirostris* of initial mean weight 0.7 g grew rapidly in the next experiment, however (Table 4). Mean weight more than doubled among animals fed the best Diets 29, K, and 1. It is again of interest that those three feeds contained the greatest amount of squid meal, and that the poorest growth and survival were found among animals fed diets in which soy was increased in relation to the squid meal.

The results suggested that *P. stylirostris* may have different nutritional requirements depending on the size of the shrimp, but experiments for longer periods are needed to confirm these results.

It also appeared that either the level or composition of the vitamin mix is significant. Neither Diet 23 nor 24, each with 0.5% vitamins, produced growth as good as Diet K containing 2% vitamins. However, vitamin C, choline, and inositol were not present in the mix used at 2% level but were so in Diet 24. Thus, the question of the significance of these vitamins must be elucidated in respect to the other dietary components.

In this series consistently poor growth was reported with the commercial ration 11-B, perhaps due to the lower level of protein (19.9%). This level is about 10% less than any other of the diets tested, which contain 30-36% protein, the level that most authors report as optimal for these two species (Colvin and Brand 1977; Forster 1976; New 1976).

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